(12) UK Patent Application (19) GB (11) 2 290 707 (13) A

(43) Date of A Publication 10.01.1996

(21) Application No 9412983.0

(22) Date of Filing 28.06.1994

(71) Applicant(s)
Georgi Stankov
Rentamstrasse 10, D-85551 Heimstetten,
Federal Republic of Germany

(72) Inventor(s)
Georgi Stankov

(74) Agent and/or Address for Service
Frank B Dehn & Co
Imperial House, 15-19 Kingsway, LONDON,
WC2B 6UZ, United Kingdom

(51) INT CL^6
A61K 31/35

(52) UK CL (Edition O)
A5B BHA B170 B180 B21Y B216 B823 B825 B826 B828 B829
U1S S1313 S1318 S2410 S2412 S2413 S2416 S2418

(56) Documents Cited
EP 0542630 A2 WO 93/03737 A1 WO 91/13595 A1
WO 89/03673 A1
Chemical Abstracts 113:144908 & J.Med.Virol. 31(2), pages 155-60 (1990) Chemical Abstracts 102:145993 & Mol.Genet.Mikrobiol.Virusol 5, pages 41-6 (1984)
Chemical Abstracts 102:39536 & Antiviral Res.4(5), pages 231-44 (1984)

(58) Field of Search
UK CL (Edition M) **A5B BHA BJA**
INT CL^5 **A61K 31/35**
ONLINE DATABASES: WPI, CAS-ONLINE

(54) Pharmaceutical uses of Amphotericin B

(57) The use of Amphotericin B for preparing a orally, intranasally or topically applicable pharmaceutical composition useful for the stimulation of bodycells of a human patient in the treatment or prevention of a variety of diseases; e.g.:-

a) viral or bacterial infections,
b) diseases associated with an impaired function of XC class I and II and/or other integral proteins,
c) cholesterol associated diseases,
d) cancer,
e) ulcers,
f) degenerative diseases, or

useful in wound-healing.

GB 2 290 707 A

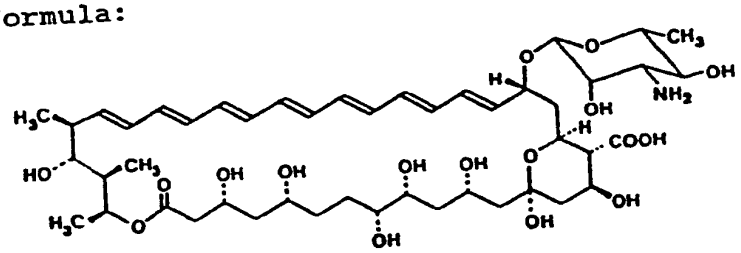
M/35088

NOVEL TYPES OF MEDICAL USE FOR AMPHOTERICIN B

The present invention refers to the use of Amphotericin B (Amp) for preparing a orally, intranasally or topically applicable pharmaceutical composition useful for the stimulation of bodycells of a human patient in the treatment or prevention of a variety of diseases.

BACKGROUND OF THE INVENTION

Amp belongs to a family of compounds designated as polyene macrolides, and was isolated in 1953 as a metabolite of Streptomyces nodolus. Amp is characterized by the following structural formula:



Because of its π-electron systems, comprising of seven conjugated double-bonds Amp can be designated as heptaene macrolide.

Although Amp has been well-known for several decades, its spectrum of therapeutical in vivo applications is practically limited to the use as antimycotic agent. Amp shows a broad range of antimycotic activity. Depending on the dose applied, Amp shows either a fungistatic or a fungicidal effect. It is assumed in the art, that Amp and other polyenes like Nystatin (Nys) exert their antimycotic effect by forming a complex with ergosterol, a membrane component of yeasts. As a consequence thereof the membrane is disrupted and the yeasts are lysed. The yeast metabolism is inhibited by the enhanced permeability of ions, water and other soluble components.

For treating superficial mycotic infections, Amp is applied topically onto the infected area. Amp may also be administered

orally in solid or liquid form in order to treat local, i.e. intestinal or oral, mycotic infections. Amp is also given i.v. for systemic mycosis

Up to the present date it is assumed that polyenes, like Amp, are not or not sufficiently resorbed upon oral, intranasal or topical administration [Bennet,J.E. in Goodman and Gilman's, The Pharmacological Basis of Therapeutics, 8th Edn., 1165-1181 (1992); Arzneimittel Fortschritte 1972 - 1985, Kleemann et al.,Editors, Verlag Chemie, Weinheim Germany, 1176-1184]. In particular it is assumed that Amp is almost unabsorbed in the gastrointestinal tract. This general prejudice in the art may be regarded as the main reason, why the practical therapeutical use of Amp is predominantly limited to the treatment of mycotic infections. The i.v. use of AMP is restricted to the treatment of systemic mycosis in neutropenic patients.

Moreover, it is well estabished that Amp and other polyenes cause severe local inflammations upon i.m. administration. In addition, i.v. administration of Amp is not applied over a longer period of time, because of severe side effects. These are further reasons for the limited therapeutical use of Amp.

Amp liposome preparations have been recently developed for i.v. administration to treat systemic mycosis. Liposome preparations have been found to reduce the toxicity of Amp. However, experimental results indicate that these preparations are less effective in the treatment of yeasts. Amp and other polyenes exert their effects on cell membranes (see below). If they are commercially associated with liposomes to which they strongly attach, they cannot interact with biologic membranes and will have poor pharmacological effects. Therefore, no liposomal preparations are intended in this patent.

SUMMARY OF THE INVENTION

The problem of the present invention is, therefore, to broaden the therapeutical spectrum of Amp by providing new application forms and indications of medical use for this compound.

This problem could be solved by the surprising finding that Amp and functional derivatives thereof, like C_1-C_5 alkyl esters thereof, as for example the methyl, ethyl, n- and i-propyl, n-, i- and t-butyl, or n-, i- or neopentyl esters, are almost completely resorbed in vivo upon oral or intranasal administration and exert pronounced systemic effects based on the stimulation of bodycells. Moreover, it surprisingly turned out that Amp can be given orally in 10 to 20-fold higher concentrations than i.v. during chronic treatment without the undesirable side effects known from the i.v. administration of Amp. The same applies to the intranasal route.

Unexpectedly it was also observed that Amp or functional derivatives thereof can be applied topically for the treatment of diseases different from local fungal infections.

According to the present invention, it was surprisingly established that the therapeutical applicability of Amp is not limited to its use as locally and selectively active antimycotic agent as conventionally believed. It emerged that Amp also exerts stimulatory effects on a great variety of bodycells, either normal or damaged by illness, of a subject. This unexpected finding opens the door for a great variety of novel therapeutical in vivo applications for Amp.

Specific preferred embodiments of the invention can be taken from the attached claims.

The great variety of therapeutic applications disclosed for the first time by the present invention is deemed to be based on the novel pharmacological effects of Amp (see below) which in

turn are linked to the unique chemical polyene macrolide structure of Amp. The specific supramolecular structure of Amp exhibits unique physico-chemical properties beyond the covalent bonding that have not been appreciated by current pharmacology and biochemistry. The present invention is based on a completely novel bioenergetic understanding of the pathogenesis of various diseases in conjuction with the unique macrolide properties of Amp that are common to nearly all polyene macrolides.

DETAILED DESCRIPTION OF THE INVENTION

I. General Considerations

1. The structure of Amphotericin

Amp, like all polyenes, consists of two different moieties, namely a macrolide ring and an amino sugar. The amino sugar mycosamine (3-amino-3,6-dideoxy-D-mannose) is glycosidically attached to the macrolide ring. The macrolide ring of carbon atoms is closed by the formation of an internal ester or lactone. It builds a stable rod-like structure with a hydrophobic polyene chain on one side and an opposite hydrophilic side, built by hydroxyl groups. This provides Amp with amphipathic features. Amp avidly self-assembles with other lipids in aqueous solutions to form reversible complexes in lipid bilayers.

The conjugated double-bonds of Amp are in the trans-position. The sugar moiety is bonded at the one end of the macrolide rod and carries a primary amino group. At the same end, a carboxyl group on the main macrolide ring is present. A single strongly polarized and hydrophilic hydroxyl group is positioned at the other end of the macrolide rod. The amphoteric character of Amp is co-determined by these groups. The occurrence of a sugar moiety with an amino group and a carboxyl group on the same end of the macrolide rod and a hydroxyl group on the other end of

the rod, determine the dipole character of the Amp and the orientation of insertion in the lipid membranes. There is a strong evidence that the sugar moiety and the carboxyl group are on the extracellular side of the membrane just as in all membrane-bound glycoproteins, while the hydroxyl group is situated towards the cytoplasmic side of the membrane.

Amp exhibits structure and properties very similar to those observed in transmembrane α-helices of integral proteins which are responsible for cell regulation (see I. General Considerations). The carboxyl group on the macrolide ring may act as an electron donor. The amino group on the sugar moiety may act as an electron acceptor. The hydrophobic π-electron system is inserted into the membrane and interacts predominantly with cholesterol, but also with other membrane phospholipids. The polyene chain is central to the energy transduction across the membrane. The polyene chain may translocate electrons (i.e. negative charges) or positive charges between donor and acceptor groups. It is assumed that electron transport along this structure is associated with a charge transport across the plasma membrane. In the context of the present invention, it was observed that all integral proteins such as receptors, ion channels and ion- motive ATPases carry in their structure specific groups that can be defined as electron donor(s), electron acceptor(s) and delocalized π-electron system(s). The latter transfer electrons between the former two. During this quantum process, all integral proteins undergo conformational changes and enhance the permeability of biologic membranes for ions and substrates (see also the general considerations below). This novel bioenergetic analysis of the chemical structure of Amp reveals that the drug possesses the necessary supramolecular structure to exhibit similar properties as those of physiological integral proteins in eukaryotes.

The ionophoric properties of Amp are a well-established fact. Several models have been proposed, the de Kruiff's model being the most widely accepted. This hypothetical model is based on

the steric structures of polyenes and cholesterol. For Amp, it postulates a complex of 8 polyene molecules associated in a ring with 8 cholesterol molecules. The inside of the ring is hydrophilic, while the outside of the ring is hydrophobic. While the immediate association of Amp with cholesterol in aqueous solution is well-established, there is no experimental evidence that confirms the de Kruiff's model. This model is static and cannot explain satisfactorily the ionophoric properties of Amp, nor can it explain the various pharmacological effects that we observed with this drug. This can be done by introducing a novel concept based on quantum mechanics (see below).

2. Bioenergetical considerations

It is not intended to limit the present invention by the following more general considerations. However, it is believed that the following considerations provide a suitable basis for an understanding of the present invention, i.e. the novel pharmacologic effects observed with Amp. Moreover, these considerations should allow a skilled person to modify or extend the specific teaching presented herein, without a departure from the scope of the invention.

Basically, these considerations concern the mechanisms of dislocated energy coupling across two biologic membranes in the cell. The energy for cell metabolism and regulation is mainly produced in mitochondria during oxydative phosphorylation (OP). As endproduct thereof electrons are obtained. These electrons are accepted by the π-electron systems of the prosthetic groups of the respiratory chain, including the proton-driving pumps. The delocalized π-electron system may harbour unpaired electrons in localized, energetically favourable positions which then may acquire a negative charge. When an unpaired electron is transferred to an adjacent electron acceptor (O_2 is the ultimate electron acceptor in OP), its position may acquire a positive charge that can move along the π-electron system. In this charge transfer, protons are expelled from the matrix

(negative side) to the cytosolic side of the mitochodrial membrane (positive side) and build a proton gradient across the mitochondrial membrane.

This mitochondrial electrochemical potential (EP) drives the ATP synthesis (F_0/F_1 ATPase) in a delocalized manner. ATP is central to most metabolic pathways in the cell. A large portion of ATP is consumed by the ion- motive ATPases such as the Na^+K^+-ATPase to maintain the EP of the plasma membrane. A positive change in the mitochondrial EP (= repolarisation) means an increase in the production of ATP. There is a dynamic balance between EP and ATP production. The redox potential of the respiratory chain that produces electrons is coupled thereto in a delocalized way. This coupling is confirmed by various chemiosmotic experiments. When the mitochondrial EP is repolarized, more ATP will be generated. To maintain the balance between the redox potential and the augmented ATP production, OP is enhanced to generate more electrons and re-establish the balance between the redox potential and the ATP production. ATP has a high affinity to F_0/F_1 ATPase and binds tight at equilibrium. When EP is applied to a mitochondrial membrane, carrying F_0/F_1 ATPase, a strong disequilibrium is created along the F_0/F_1 structure. This disequilibrium is the result of the electric field created by the mitochondrial EP that is of the magnitude of 10^8 Vm^{-1}. The F_0/F_1-ATPase is an integral protein with a strong dipole moment. Repolarization augments the vector of the electric field that activates the F_0/F_1-ATPase and induces conformational changes on its structure. This reduces the affinity of F_0/F_1-binding sites to the ATP molecules so that the latter dissociate. ATP is set free in a delocalized way when the mitochondrial EP is repolarized.

It is a well-established fact that all cells exhibit EP across their plasma membranes, ranging from 60 to 110 mV, depending on the cell type. The plasma membranes are negatively charged inside and positively charged outside. Thus, both the

mitochondrial and plasma EP have the same direction. The two membranes can be regarded as serially coupled capacitators and their EP, and their electric fields, can be added. The EP of other organelles are serially coupled to the plasma EP and parallel to the mitochondrial EP.

For the cytoplasm, the principle of electroneutrality is valid. The principle of electroneutrality reaffirms the principle of delocalized energy coupling. The principle of electroneutrality states that any macroscopic region of a solution must have an equal number of positive and negative charges. Thus, the charge of the mitochondrial positive side is identical with the charge of the negative side of the plasma membrane. Any depolarisation of the plasma EP means an increase in the charge of the negative cytosolic side. The positive side of the mitochondrial membrane will also exhibit an equivalent and immediate increase of its charge, when the cell is depolarized.

Consider a plasma EP = - 60mV as observed in leukocytes. A depolarisation of about 20 mV will lead to a plasma EP = -40mV. The mitochondrial EP is repolarized from 200 mV to 220mV in a delocalized way when the plasma EP is depolarised from -60mV to -40mV. Thus, depolarization of cells leads to repolarization of mitochondrial EP and generates more ATP.

A depolarisation of plasma EP means that the cell disequilibrium is displaced towards equilibrium and must be re-established, so that the cell can be activated again. The plasma EP thus represents the stored energy immediately available for biologic regulation. This energy is stored by the work of ATPases, mainly by the Na^+K^+-ATPase that expels the two ions against their gradient in order to maintain plasma EP. The hydrolysis of ATP drives the Na^+K^+-ATPase and maintains the cell disequilibrium. We showed that a depolarisation of plasma EP leads to repolarisation of mitochondrial EP and to an increase in the ATP synthesis. This increase in ATP is consumed by the Na^+K^+-ATPase to re-establish the initial plasma EP, by pumping Na^+ and K^+ against their gradients (K^+-influx and

Na^+-efflux). When the plasma EP is repolarized, the opposite situation is observed.

The plasma EP is coupled to the mitochondrial EP in a delocalized way. This also applies to all other intracellular potentials and compartments. The self-organisation of cell metabolism, including the regulation of the genetic code and protein synthesis, is based on this bioenergetic mechanism. It is assumed that cells are dissipative systems that create their own disequilibrium which in its turn drives cell metabolism. Regulation of cell metabolism is achieved by modulating this disequilibrium. The interaction between plasma EP and mitochondrial EP is essential, because most of the metabolic energy is generated in mitochondria. Such interaction occurs simultaneously and immediately also between plasma EP and the EP of any intracellular membrane compartment (e.g. endoplasmatic retuculum, Golgi, vesicles, etc.). Thus, plasma EP and mitochondrial EP, as well as any intracellular EP, represent the stored interconvertible energy that is at any time at the disposal of the cell to drive its metabolism.

The modulation of plasma EP through depolarisation and repolarisation is the fundamental mechanism of biologic regulation that represents a process of self-organisation. It was found that all integral proteins (receptors, channels and ion-motive pumps) and all known physiological factors such as neurotransmitters, hormones and humoral factors participate in the self-regulation of cells, either by causing depolarisation or repolarization.

The electron donor(s) of an integral protein emit electrons that are temporarily stored in the π-electron system(s). The unpaired electrons build a delocalized π-electron anion. They dwell preferentially in localized energetically favourable positions called "midgaps" that are negatively charged. A localized positive charge is formed when these unpaired electrons are translocated to the electron acceptor(s) upon activation of the integral protein. The positive charges are

dynamically translocated across the membrane along the electrochemical gradient or against it, depending on the type of the integral protein. This quantum process induces specific conformational changes in the structure of the integral proteins and enhances the permeability of the membrane for ions or substrates. This process is called "energy transduction" (ET) and is basic to the bioenergetic concept underlying the present invention.

Based on the above considerations, it was established according to the present invention that the same quantum process occurs in the macrolide structure of Amp when the drug interacts with cell membranes. Due to the unique supramolecular structure of Amp, including an extended delocalized π-electron system, an electron donor-group and an electron-acceptor-group, the drug may operate in the plasma membrane of eukaryotes as an autonomous functional unit, sharing striking similarities with integral proteins that are basic to cell regulation. These integral proteins are of the depolarizing type and participate in the stimulation of cell metabolism, cell growth and cell proliferation, and enhance the endocytosis of other integral proteins, expressed in plasma membranes. Amp can be regarded as a supramolecular non-proteinic prototype of a depolarizing integral protein, synthesized by Streptomyces nodolus. Amp is thus designated as "depolarizing ET-enhancer". This is a fundamental novel approach explaining the pharmacological effects of Amp at the quantum level. The depolarization of Amp may be discrete and may not always be measured experimentally. In vivo, cells have the propensity to compensate immediately any changes in their disequilibrium, but various cell stimulating effects of Amp are always observed at physiological concentrations.

Depolarisation and repolarisation of biologic membranes are the two fundamental mechanisms that regulate the cell. Both phenomena are the aggregated product of activation of membrane proteins whose activity is modulated by the dielectric properties of the membrane (e.g. cholesterol, degree of

unsaturated fatty acids, etc.). Depolarisation means cell stimulation such as cell growth, cell proliferation, protein synthesis, and a more dynamic exchange of integral proteins by endocytosis. Cell repolarisation means that the set point of activation is up-regulated. Cell repolarisation leads to cell maturation and cell differentiation, and a prolonged expression of integral proteins. Both depolarisation and repolarisation involve active energy transduction across biologic membranes. All physiological factors (hormones, neurotransmitters, humoral factors) are either depolarizing or repolarizing factors. They all enhance the energy transduction across the membrane.

Cells are open disequilibrium systems. The cell disequilibrium is created by the electric fields of the plasma and the mitochondrial EP which are about 10^8 Vm^{-1}. This means that there is a free supply of energy and substrates that flow in a coordinated manner into the cell during regulation. The energy flow is accomplished by the modulation of the plasma EP. The supply of substrates also follows the bioenergetic mechanisms of self-organisation. When the plasma EP is depolarized, this is mainly achieved by Na^+-influx and K^+-efflux along the gradient because these ions are mainly involved in the plasma EP. All cells exhibit Na^+-coupled import of amino acids and glucose. Recently, Cl-coupled symport of substrates has also been discovered. Hence, the transport of substrates into the cell is self-regulated during depolarisation. Depolarisation means cell stimulation, leading to increased metabolism. Automatically, a surplus of substrates is provided into the cell by Na^+-symport. During repolarisation, the opposite effects are observed. At any time, there is an optimal synchronization between energy and substrates supply on the one side and metabolism on the other side.

There appears to be a close bioenergetic link between cell disequilibrium and the genetic code. Depolarisation enhances transcription, repolarisation may inhibit or modulate it. In fact, both states correspond to different sets of transcription patterns. Dissipation of the disequilibrium stops transcription

and leads to cell death. Any impairment of the cell disequilibrium towards equilibrium imposes a strong constraint on the genetic code to counterbalance this impairment by mutation. This is the mechanism of transformation of body cells to cancer cells. This is important for the understanding of the anticancer effect of Amp. Cells with an impaired disequilibrium mutate to produce abnormal quantities of, for example, growth factors to enhance an autocrine stimulation. Transformed cells no longer depend on supracellular regulation. They are autark. In vitro they can reproduce infinitely. As in all dissipative systems, the transformation of cells is a singularity in a non-linear process.

II. Pharmacological Effects of Amp

At present, the quantum phenomena associated with the Amp structure as disclosed above cannot be measured in vivo. However, specific pharmacological effects of Amp can be expected on the basis of the above disclosed general considerations. These effects can be observed at the macroscopic level and are responsible for the therapeutic efficacy of Amp in a variety of diseases claimed in this invention.

For instance, Amp may depolarise eukaryotic cells by increasing mainly the permeability of the membrane for Na^+ and K^+. Amp causes predominantly Na^+-influx into the cell and K^+-efflux out of the cell. These ionic currents may influence the permeablity of other major ions that participate in the electrochemical gradient such as H^+, Cl^- and Ca^{2+}. This effect may be direct or indirect. Cells, incubated with Amp, experience a rapid increase in intracellular calcium, that is proportional to Amp concentration. In therapeutic concentrations, Amp stimulates all eukaryotic cells investigated so far and is thus an universal ET-enhancing drug. In the process of cell stimulation, which is effected in a localized and a delocalized manner, Amp can activate various local membrane effector systems (e.g. DAG, PIP_3-cascade, adenylate cyclase, G-proteins,

etc.). In addition, Amp stimulates amino acids and glucose transport into the cell, which is linked to Na^+-transport (symport). It can also stimulate in a delocalized way the synthesis of ATP in mitochondria and various DNA regulatory proteins and enhance protein synthesis. It also stimulates the de novo phospholipid synthesis and glycolysis. These effects are the result of the ubiquitous depolarising properties of Amp. Depolarisation is also associated with endocytosis of integral proteins as part of the self-regulation of the cell. Amp can decrease the expression of integral proteins or promote their exchange, depending on the baseline conditions of the cell in the context of self-organisation in systems, highly displaced from equilibrium. This pharmacological effect is useful in the treatment of many diseases as claimed by the present invention.

It is a well-established dogma that polyenes like Amp are specifically fungicidal by forming complexes more avidly with ergosterol in yeasts than with cholesterol in human cells, but this notion has not been experimentally verified. On the contrary, Amp readily assembles both with cholesterol and with ergosterol. The cytotoxic effect of Amp in yeasts is due to an excessive depolarisation that dissipates the electrochemical gradient of yeasts and induces lysis. This effect, however, can be observed also in eukaryotes when they are incubated with high Amp concentration for a long period of time. In lower, therapeutic concentrations, Amp stimulates both yeasts and eukaryotes. We suggest that the elimination of yeasts in the human organism is achieved not by direct elimination of yeasts, as conventionally believed, but indirectly by stimulating the overall resistance of the body cells i.e. by improving their disequilibrium. In eukaryotes, there is a strong dissociation between cell stimulation and lytic effects. In concentrations up to 50-100μg/ml, Amp stimulates most eukaryotic cells, but does not lyse them. Cell lysis is observed in concentrations above 100 μg/ml, only when the cells are incubated for 24 hours or longer. Eukaryotes have the propensity to recuperate below this time of incubations. This propensity is less pronounced in

yeasts. Amp enhances cell stimulation at concentrations of 10-20 µg/ml. This is equivalent to a total daily dose of about 700-1500 mg Amp in adults (approx. 10 to 20 mg/kg body weight). This therapeutic range has been experimentally confirmed to be safe and effective in the treatment of a variety of diseases. Thus, the lytic concentrations of Amp exceed more than 10 times the necessary therapeutic concentrations in humans.

As already mentioned, polyenes are believed to be unresorbed or very poorly resorbed after oral administration. This belief is based on the low plasma concentrations measured after the oral administration of Amp and Nys. These polyenes are commercially available for the therapy of yeast infections. However, the kinetic behaviour of these drugs is very poorly investigated. For example, the kinetics of Amp, given i.v., is based on the results of only two patients [A.J. Atkinson & J.E. Bennet, in Antimicr. Agents & Chemoth. (1978), Vol. 13, p. 271-276].

The present invention is also based on the surprising finding that Amp is resorbed to a large extent after oral or intranasal administration.

The resorption of lipophilic drugs such as Amp and other polyenes cannot be determined from their plasma concentrations as they bind to plasma membranes and are distributed in the so-called deep compartment. Such drugs appear only in negligible concentrations in the plasma and are almost completely bound to lipoprotiens or other proteins. For such drugs, the resorption can be determined by the observation of systemic effects, based on their pharmacological profile. When these effects disappear during dechallenge and appear after rechallenge this is generally accepted as strong evidence for their gastrointestinal resorption and their bioavailability in the organism. Another possibility is to assess the magnitude of the systemic effects in dependence of the administered dose. We conducted dechallenge-rechallenge experiments or varied the dose in patients with various indications and concluded that Amp, Nys and other polyenes are largely resorbed from the gut

and exhibit pronounced systemic effects: These effects can be summarized as follows: a) dose dependent increase in vaginal discharge after 2 weeks of treatment; b) dose-dependent increase in mucous secretion in the airways after approx. 8 weeks of treatment; c) increase in bile secretion and disappearance of gallstones; d) increase in the prostaglandin secretion after 1 week of treatment; e) dose-dependent decrease in Ch plasma levels after 2 to 4 weeks of treatment. f) decrease of the size of the prostatic gland after 4 weeks of treatment. g) improvement of the skin turgor. h) lowering the mortality in severe trauma patients with sepsis, etc. None of these effects can be explained if we assume the traditional view that Amp, Nys and other polyenes are not resorbed after oral administration.

As there is no evidence that Amp or other polyenes are degraded in the digestive tract, the concentration of Amp and Nys was measured in the faeces of patients treated with these drugs. The total amount found in the faeces was less than 1% of the administered dose. This was interpreted as a strong evidence that Amp, Nys and all other polyenes are almost completely resorbed from the gastrointestinal tract.

Because of its very high affinity for cholesterol and cholesterol-derivatives, Amp binds avidily to the bile acids which facilitates its resorption so that the drug is more or less completely resorbed from the intestinal tract. Due to the amphipathic character of Amp and its high affinity for cell membranes, it associates with adjacent cell membranes immediately after resorption and does not appear in blood plasma. Oral Amp distributes immediately in the so-called deep compartments, predominantly in liver, lymphatic organs and spleen. After resorption from the intestinal mucosa, Amp is transported via the lymphatic pathways to the liver and other secondary lymphatic organs where it interacts with cells of the immune system. Due to its lipophilicy, orally administered Amp does not reach in sufficient concentrations vital organs such as kidney, heart and lungs. This is obviously also true for

intranasally administered Amp. Therefore, oral and intranasal Amp does not cause serious adverse events that are commonly observed after i.v. administration. This explains the extraordinary safety of Amp when administered orally compared to the high toxicity of this drug when administered intravenously. This dichotomy in the safety profile, determined by the pharmacokinetic property of Amp, has lead to the wrong notion, persisting for the last 40 years, since Amp has been on the market, that it is not resorbed orally. For this purpose, Amp is given orally only for short-term gut decontamination, while its long-term stimulating effects on body cells which become clinically manifest after approximately three months of chronic treatment are not known.

In summary, the depolarizing effect of Amp causes the stimulation of cell growth, proliferation and various other metabolic activities such as protein synthesis, endocytosis and increased turn-over of receptor expression on cell membranes, increased turn-over of phospolipid membranes, etc.

Preferably, Amp preparations should not contain any other active pharmacological ingredients, except the necessary galenic ingredients for the preparation of tablets, capsules, ointments or other galenic forms for oral, intranasal or topical administration, because they may interfere with the active ingredient Amp.

III. Novel In-Vivo-Indications for Amp

1. The Use of Amp in the Treatment of Sepsis, Bacterial and Protozoal Infections and for Wound Healing

In order to avoid sepsis, Amp may be orally administered for selective decontamination of the digestive tract (SDD) in ICU-patients (ICU = intensive care unit). A major component of an SDD regimen is either Amp or Nys, given orally (via tubus), immediately after delivery to the hospital and for the duration

of the ICU stay. Systemic i.v. antibiotic treatment is given for early prophylaxis during the first 4 days after trauma and thereafter at the discretion of the physician. In a trial in severe trauma patients conducted by us in 20 ICU in 8 West European countries, there is a strong statistical evidence, gained in an interim analysis from approximately 400 patients, that SDD with oral polyenes (Amp and Nys) can reduce mortality. As no yeast infections have been reported in the SDD group, it has been concluded that the effect of Amp and Nys is definitely not antifungal, but should be attributed to their modulating effect of plasma EP that enhances by energy transduction. These effects stimulate the bodycells, like cells of the immune system, and enhance the natural resistance of the organism.

A number of clinical trials, using topically applied Amp have been performed exploratively. During these experiments it was observed that Amp enhances the healing of wounds, burns, and injuries of the skin and the mucosa (e.g. recurrent aphthous stomatitis, subprosthetic stomatatis). The healing effects were dramatic and incomparable to anything described in the literature so far. In other clinical studies, it was observed that Amp is also effective in the topical treatment of acne, neurodermatitis, decubitus and ulcus cruri. Amp improved the symptoms of chronic cold-agglutinin disease, etc. All of these surprising effects can be explained satisfactory only by considering the modulating effects of Amp on the cellular disequilibrium at the quantum level.

Oral Amp or Nys were found to be effective in perioperative prophylaxis. An analysis of the results from a large controlled clinical trial with hospitalized patients, undergoing major abdominal surgery suggests that the postoperative infection rate has been lowered to virtually zero from 2 to 8 % (depending on the type of surgery) before Amp or Nys were applied. Patients received Amp or Nys for SDD approx. 1 week before operation.

Based on the cell-stimulating in vivo effects of Amp, the drug

may also be applied for the treatment of infections with protozoan (leishmaniasis, malaria, etc.) and bacterial infections caused by a gram-negativ, gram-positive, aerobic or anaerobic bacterium.

As examples for gram negative bacteria acinetobacter species (sp.), citrobacter sp., enterobacter sp. escherichia coli, haemophilus influenzae, klebsiella sp., moraxella sp., neisseria gonorrhoeae, neisseria meningitis, proteus sp., providencia sp., pseudomonas sp., salmonella sp., serratia sp., shigella sp. may be mentioned.

As examples for gram-positive bacteria staphylococcus (staph.) aureus, staph. epidermidis, other staph. sp., streptococcus sp. may be mentioned.

Typical examples for anaerobic bacteria are bacteroides sp., clostridium sp., fusobacterium sp. peptococcus sp., peptostreptococcus sp., veillonella sp.

2. <u>The Use of Amp in the Treatment of Virus Infections</u>

a) AIDS

The pathogenesis of AIDS is not known at present. Even the viral origin of the disease is questioned. The pathogenesis of AIDS is revealed in the light of the general considerations on which this invention is based. In the context of this invention, the bioenergetic explanation of the pathogenesis of AIDS provides a stringent and comprehensive model that can be applied to all viral infections. Based on this novel approach, the therapeutic effects of Amp in the treatment of HIV-1 and other viral infections claimed in this invention can be explained.

HIV is enveloped by a lipid bilayer, containing two glycoproteins: gp41 that spans the membrane and is bonded to the extracellular gp120. In HIV-infected cells, these proteins

are expressed on the cell membrane. Nonpolymorphic determinants of the MHC class II, particularly HLA-DR and HLA-DQ share structural homology with the gp41-gp120 complex of HIV-1 and antibodies to these HIV-proteins can cross-react with HLA class II molecules. Antibodies against MHC class II are found in serum of HIV-patients. Anti-gp120 antibodies are also detected on CD4 T lymphocytes in AIDS patients. Perturbations of T cell subgroups bearing specific variable ß regions are observed in HIV-patients. Apoptosis of T cells in lymphatic organs is made responsible for the depletion of CD4. Normally, only 1 in 10000 to 1 in 1000 CD4 cells express the virus. Other viruses can up-regulate the expression of HIV (gp120). Repolarizing cytokines as TNF-ß, TNF-α, INF-gamma, etc. can stimulate HIV replication, while depolarizing cytokines such as INF-α, INF-ß, etc. suppress the effect of HIV infection. Although the peripheral CD4/CD8 ratio is considered pathognomonic for the degree of HIV-infection, now it is evident that the disease progresses continuously in the lymphatic organs so that there is actually no incubation time. The late phase with viraemia reflects the decompensation of the lymphatic system. The dendritic network is destroyed and infected CD4 cells are released in the circulation.

Bioenergetic considerations are virtually unknown to current virology. The role of viral proteins expressed on the membrane of infected cells has never been associated with the ability of the virus to replicate or destroy host cells as in the case of AIDS. Viral glycoproteins expressed on membranes have been only considered as markers for infection. This is so, because the delocalized bioenergetic coupling between membrane proteins, responsible for the cell disequilibrium, and the DNA replication is unknown. The central role of the delocalized energy-coupling for the virus replication becomes evident in the context of the present invention.

According to the general considerations disclosed above it is assumed that the expression of the gp120-gp41-complex is a prerequisite for the replication of proviral DNA. The complex

alters the cell disequilibrium of the infected cell in a specific way so that the proviral DNA can be replicated. The virus thus makes use of the bioenergetic coupling between cell regulation and gene expression. The genetic mechanisms of HIV, including reverse transcriptase, the building and insertion of proviral DNA in the genom, the gag, pol and env genes, can only operate when the gp120-gp41-complex is expressed. gp41 is the transmembrane part of the complex, while gp120 corresponds to the variable domain of the MHC class II molecule. The infected CD4 cells are depolarized/activated in a specific way in the lymphatic nodes and other secondary lymphatic organs and replicate the virus. Thus, the HIV-virus uses the physiological mechanisms of the immune system to replicate. This is possible because of the structural similarity between the gp120-gp41 complex and the MHC class II. The MHC molecules on antigen-presenting cells (APC) are central for the stimulation and selection of immunocompetent B- and T- cells. The viral gp120-gp41-complex biases the immunologic selection that determines self-tolerance and allo-reactivity. Some infected CD4 cells are excessively stimulated by antigen-presenting cells and die by apoptosis, while other cells are optimally stimulated and replicate the HIV-virus. These cells release the virus and also die. A third subgroup of cells is not stimulated at all and becomes anergic. These phenomena are all observed in AIDS patients, but could not have been explained until now.

Treatment of AIDS with Amp is a novel therapeutic approach. It is based on the early stimulation of the body and the immune system immediately after infection (occurrence of seropositive reaction). The treatment should be chronic, probably life-long as the virus cannot be eradicated from the cells. Amp stimulates all immune cells and especially the T cells by depolarizing them. Depolarized infected cells do not express the gp120-gp41 complex. The proviral DNA can not be replicated by a specific delocalized coupling. At the same time, the disequilibrium of these cells is maintained so that they can recruit their own DNA repair mechanisms to eliminate the HIV virus. The replication of the HIV-virus requires optimal

intracellular conditions and, above all, the expression of the viral gp120-gp41 complex. The expression of gp120-gp41 correlates with the infection rate of children born by HIV-mothers and with the outcome of the disease. An inhibition of the gp120- gp41 expression precludes the depletion of CD4 cells by the above mentioned mechanisms. Stimulated cells have a higher likelihood of overcoming the virus infection.

The rationale behind the treatment of AIDS patients with Amp may be summarized as follows: It stimulates the infected cells and suppresses the expression of the gp120-gp41 complex. Amp inhibits the depletion of CD4 cells and of other immunocompetent cells in the lymphatic organs, enhances the repair mechanisms of infected cells to eliminate the virus and prevents the biasing of the processes of self-tolerance and allo-reactivity by inhibiting the expression of the gp120-gp41 complex.

As most viruses are found intracellularly in the organism, the former mechanism of action seems to be paramount. The antiviral and cell-stimulating effect of Amp is basic for the treatment of various other diseases as claimed in this invention. For example, treatment of the following infections is indicated:

b) Herpes Simplex Virus (HSV) Infection

Amp inhibits in cell cultures HSV I and HSV II at concentrations between 3 and 25 µg/ml. Moreover, a rapid improvement is observed after topical administration of Amp to patients suffering from labial HSV infections. Frequent applications increase the effectiveness of the Amp treatment.

c) Herpes Zoster Varicella (HZV) Infection

Amp inhibits in cell cultures HZV at concentrations between 3 and 25 µg/ml.

d) Hepatitis B Virus (HBV) Infection

Amp inhibits dose-dependently the production of hepatitis B surface antigen (HbsAg) in human hepatoma cell line PLC/PRF/5.

e) Vesicular Stomatitis (VS), Influenza and Reuscher Leukemia Virions

Polyenes like filipin, Amp and Nys inactivate these virions in vitro by altering the structure of the lipid envelope.

f) Other Virus Infections:

Amp inhibits in vitro also Sindbis virus and vaccinia virus.

g) Recurrent Aphthous Stomatitis (RAS)

The pathogenesis of RAS is not clear, but there is substantial clinical evidence that it can be triggered by various virus infections. Patients with RAS have been treated topically with Amp. Pain was relieved immediately after the first application. Depending on the diameter of the lesion, RAS disappeared within 24 to 48 hours. None of the patients experienced RAS for more than 4 days. There is evidence that the recurrence rate can be decreased, too.

h) Based on the data and the considerations mentioned above, the use of Amp in the treatment of all viral infections should be beneficial, such as (but not exclusively):

Infections with picornaviridae (e.g. poliovirus), caliciviridae (e.g. Norwalk virus), togaviridae (e.g. rubella virus), flaviviridae (e.g. yellow fever virus), coronaviridae, rhabdoviridae (rabies virus), filoviridae (Marburg virus), paramyxoviridae (measles virus), orthomyxoviridae, bunyaviridae (e.g. California encephalitis virus), arenaviridae (lymphocytic choriomeningitis virus), reoviridae (e.g. rotavirus), retroviridae (e.g. HIV-1), hepadnaviridae (e.g. hepatitis A and hepatitis B), parvoviridae (e.g human parvovirus B-19), papovaviridae (e.g. JC virus), adenoviridae (e.g. human

adenovirus) herpesviridae, poxviridae, Epstein-Barr virus (e.g. infectious mononucleosis), cytomegalovirus.

3. The Use of Amp in the Treatment of Diseases Associated with an Impaired Function of MHC Class I or II (= HLA-Association) and/or of Other Integral Proteins

In the context of the general considerations, it was observed that many diseases are associated with an impaired function of the ubiquitous MHC (Major Histocompatibility Complex) class I or II. The defects involve the binding of self-peptides or allo-peptides and are genetically pre-determined (HLA-association, HLA = Human Leucocyte-Associated Antigen). This fact will be illustrated when the pathogenesis of selected diseases will be discussed below. Deficient MHC molecules lead to an impaired cell disequilibrium that can affect virtually every type of body cells. Sometimes this impaired disequilibrium may be additionally aggravated by other integral proteins that are also deficient. In these cases, the cell disequilibrium is displaced towards equilibrium. Amp acts as an autonomous depolarizing ET-enhancer and improves the cell disequilibrium of various tissues. This pharmacological effect was shown to be beneficial in many diseases of the above mentioned type.

The pathogenesis of rheumatoid arthritis (RA) and multiple sclerosis (MS) have been chosen as typical examples for a common pathogenetic model that applies to diseases associated with an impaired function of MHC Class I or II (= HLA-Association) and/or of other integral proteins. The aetiology of these type of diseases is unknown at present. This is the first stringent explanation of their pathogenesis that is basic for the understanding of the therapeutic effects of Amp.

a) Rheumatoid Arthritis (RA)

A restricted set of genetically determined MHC class II molecules strongly predispose to RA. The LA locus occupies a small segment on a single chromosome. Increased risk for RA is associated with HLA-DR and especially with mutations of specific amino acids (glutamine and lysine residues at position 70/71 on the HLA-DRβ1 chain). These findings are analogous in collagen arthritis and in myelin-basis-protein-induced experimental allergic encephalomyelitis, which show close association between disease susceptibility and specific MHC class II molecules. Their exact structure has been recently elucidated. All 15 hydrogen bonds in the MHC class II-groove involve specific amino acids that act either as electron acceptors, electron donors or carry π-electron systems and thus participate in the quantum processes of energy transduction described for Amp and other integral proteins. Mutations of these amino acid residues lead to a deficient binding of self-proteins or allo-proteins and thus impair the processes of self-tolerance and allo-reactivity.

In RA, APCs expressing MHC class II of the HLA-DR allele, cannot effectively bind type II collagen that is produced from the incessant bone remodelling in the organism and present it to the T-cells. The T-cell receptor interacts with the MHC-molecule, only when it is loaded with a self-peptide. In the process of this MHC-restricted interaction, which represents an energy transduction across the T-cell membrane, the T-cells are stimulated (through depolarization and secretion of lymphokines from both the T-cells and the APC cells) and are selected. This is the bioenergetic mechanism of developing self-tolerance and allo-reactivity of T-cells in the immune system. The T-cell receptors can not be activated appropriately and the selection of the T-cells is impaired. In this case, inefficient subtypes of T-cells are produced.

In RA, CD4 cells are found in large quantities at the site of inflammation. This indicates that the CD4 cells are functionally impaired. This is a common feature for most cell-mediated autoimmune diseases. Rheumatoid factors, mainly

autoantibodies of the IgM isotype are regularly found in RA-patients. Ig production of synovial B cell populations in RA are distorted towards anti-type II-collagen activity. This indicates that collagen II cannot be bound and presented adequately by MHC class II expressing cells. Thus, the stimulation of APC (almost all cells seem to present antigens) and of B-, and T-cells by Amp improves their activity and repairs the selection for self-tolerance and alloreactivity in secondary immunologic organs. In the early stage of the disease, a complete remission is possible. Treatment should be chronic. Our experience shows that positive effects may occur after 3 months, but usually they are observed after 6 months to 1 year or more, depending on the stage of the disease. Recommended mean daily oral dose is 500 to 2000 mg, preferably 1000 mg Amp. The dosage may vary, depending on the symptoms.

b) Multiple Sclerosis (MS)

MS is characterized by patches of demyelination of CNS, resulting from an antiinflammatory process. The disease has a relapsing and remitting course that is representative for chronic diseases. As in RA, MS involves CD4 cells, B lymphocytes and macrophages at the site of inflammation. Specific MHC class II alleles (DRw15 and DQw6) are associated with an increased risk of MS, which is consistent with the hypothesis that certain HLA alleles exhibit impaired binding of specific self-proteins. The latter cannot be adequately presented to B- and T-cells so that no adequate stimulation and selection is possible. This impaired function of the MHC class II alleles biases the self-tolerance and allo-reactivity of the immune system and leads to manifestation of the disease. We treated patients with MS with Amp and an improvement of the symptoms was registered.

c) The two diseases, RA and MS, are models for a large group of diseases associated with an impaired function of MHC class I or II (HLA-association) and/or of other integral proteins that exhibit common principles of pathogenesis. These diseases can

be effectively treated with Amp by stimulating the affected cells via depolarization of their plasma EP and enhancing the energy transduction. For clarity, the diseases are sub-classified anatomically. For some diseases, a HLA-association is assumed, but not yet investigated:

Bone tissue: Ankylosing spondylitis (B27), Reiter's syndrome (B27), reactive arthritis (yersinia, salmonella, gonococcus, etc.) (B27), psoriatic arthritis (B27, Bw38), juvenile rheumatoid arthritis (B27, DRw8), rheumatoid arthritis (Dw4, DR4), osteoarthritis

Gastrointestinal: Gluten-sensitive enteropathy (DR3), other food-sensitive eneteropathies, chronic active hepatitis (DR3), ulcerative colitis (B5), acute anterior uveitis (B27), Crohn's disease, liver cirrhosis

Hematologic: Idiopathic hemochromatosis (A3, B14), pernicious anemia (DR5), paroxysmal nocturnal hemoglobinuria, cold-agglutinin-disease, cryoglobulinemia, hemochromatosis, Wilson's disease, porphyrias

Skin: Dermatitis herpetiformis (Dw3), psoriasis vulgaris (Cw6), pemphigus vulgaris (DR4, A10), Behcet's disease (B5), systemic lupus erythematosus (DR3), endogenous eczema and other forms of atopy (e.g. neurodermatitis), various forms of allergy, Sjögren syndrome (Dw3), dermatomyositis, scleroderma, chronic vasculitis (e.g. Raynaud's syndrome), diseases of the hair root.

Endocrine and genetic disorders: Type I diabetes mellitus (DR4, DR3, DR2, BfF1), hyperthyroidism (B8, Dw3), hyperthyroidism in japanese (Bw35), adrenal insufficiency (Dw3), subacute thyroiditis (de Quervain) (Bw35), Hashimoto's thyroiditis (DR 5), congenital adrenal hyperplasia (Bw47), distress syndrome, chronic fatigue syndrome, postmenopausal syndrome, primary and secondary amyloidosis, gout, cystic fibrosis.

Neurologic: Myasthenia gravis (B8, DR3), multiple sclerosis

(DR2), manic-depressive disorder (Bw16), narcolepsy (DR2), schizophrenia (A28), polyneuroradiculitis (Guillain-Barre syndrome), polymyositis, slow virus diseases of CNS, other systemic diseases of CNS (e.g. amyotrophic lateral sclerosis, other demyelinating diseases), Alzheimer's disease (see also below)

Renal: Idiopathic membranous glomerulonephritis (DR3), Goodpasture's syndrome (anti-GBM) (DR2), minimal change disease (steroid response) (B12) polycystic kidney disease (B5), IgA nephropathy (DR4), Gold nephropathy (DR3, DR4)

Infectious: Leprosy (B8, Asians), paralytic polio (BW16), IgA deficiency (DR3), sarcoidosis

Humoral factors: Genetic disorders of lymphokines, complement and other humoral factors and their integral receptors.

The following experimental results provide clinical evidence for the claimed use of Amp:

Patients with gluten-sensitive enteropathy or other food-sensitive enteropathies were treated for about 6 months with oral Amp. A pronounced improvement of the symptoms was observed after 3 months.

Children, suffering from atopic neurodermatitis were treated topically with Amp paste. Recurrent exacerbations were cured within a few days. Untreated lesions did not resolve or resolved very slowly.

Patients with chronic fatigue syndrome or postmenopausal syndrome (PMS) were treated with oral Amp for several months. Both conditions substantially improved during treatment. As Amp stimulates prostaglandin production, effects were observed in the patient with PMS after 4 weeks. Proliferous vaginal serous discharge occurred after 2 weeks in the female patient and normalized to pre-menopausal levels in the course of the study.

Dechallenge and rechallenge of Amp treatment for 1 week led to a prompt interruption of the vaginal discharge. This was interpreted as strong evidence that Amp is substantially absorbed from the gut and that it exerts systemic effects after oral administration.

Severe therapy-resistant forms of psoriasis improved significantly with 1 g daily dose of oral Amp given chronically.

4. The Use of Amp in the Treatment of Atherosclerosis (AS) and Other Cholesterol-Associated Diseases like all Types of Hyperlipoproteinemias

a) Atherosclerosis (AS)

The pathogenesis of AS is unknown. There are several concurrent hypotheses at present. We suggest a novel bioenergetic model of AS pathogenesis based on the general considerations in the present invention. The therapeutic effects of Amp can be easily explained in this context.

AS represents a fibroproliferative response, involving intimal smooth muscle cells, macrophages ("foam" cells), T lymphocytes and other morphologic changes caused by an excess of cholesterol (Ch). Ch is transported by LDL and VLDL in the organism. These large spherical particles carry Ch/phospholipids in a ratio of approx. 1:1 and apolipoproteins (apoB and apoE). Both are polymorphic glycoproteins that interact with apo-receptors on cell mebranes. All cells express apo-receptors. These are transmembrane lipoproteins of the depolarizing type. They are located in coated pits. Liver cells are most prominent in this respect. ApoB and apoE bind to their cell receptors. The cell is depolarized and LDL is incorporated into the cell by endocytosis. Endocytosis always occurs during depolarisation. Intracellular LDL is delivered to lysosomes and incorporated into newly synthesized membranes. This is an extremely dynamic process, a fact that has not been adequately

appreciated by current hypotheses.

The turn-over-rate of membrane Ch is enormous. Lymphoblasts exchange their total membrane Ch every hour. Another aspect that has not been appreciated so far, is that more than 99% of total human Ch is membrane-bound. As research has focused on the levels of circulating Ch, the LDL/VLDL ratio has been considered to be of primary importance for the pathogenesis of the disease. However, this ratio only indicates that Ch is in excess in the organism, compared to the needs of the individual cells to maintain their disequilibrium. The demand for Ch which is essential to AS is determined by the cell metabolism. In addition, the de novo synthesis of Ch occurs in the cells. Ch is also the precursor for steroid hormones in the ovary, adrenal gland, prostate and other sexual organs and into bile acids in the liver. This is an important fact that should be considered in the context of the therapeutic effects of Amp in prostatic hyperplasia, postmenopausal syndrome and the treatment of gallstones.

According to the present understanding, underlying this invention, Ch is the central molecule, regulating the dielectric properties of biologic membranes. When polyenes, like Amp, insert in the membrane, they bind to Ch. This can be observed experimentally. The purpose of this binding is physiological. As mentioned, polyenes like Amp have a strong dipole moment and increase the conductivity of biologic membranes. Amp stimulates bodycells by depolarization and enhances the Ch turn-over in biologic membranes. According to the de Kruijff's model, one Ch molecule is attached to each polyene molecule. This fits well to the general observation that the Ch: phospholipid ratio in biologic membranes is 1:1. In the presence of Amp, more Ch is bound in the plasma membrane. This leads to a decrease in the circulating Ch and prevents the occurrence of AS. This is a highly dynamic process. There is a very narrow range of Ch concentration in the membrane within which cells can effectively transduce energy across their membrane. Therefore, cells cannot

accommodate more Ch than they need. Otherwise, the cell disequilibrium will be impaired. The excess of Ch that cannot be stored in fatty tissue and in the liver circulates in plasma and deposits in the intima of blood vessels. Immunocompetent cells clear the excess of Ch ("foam" cells), but when their clearing capacity is not sufficient to eliminate the excess Ch, it deposits in the blood vessels. There, Ch produces fibroproliferative processes such as fatty streaks, fibrofatty lesions and fibrous plaques. An excess of Ch is observed in aging and should be considered responsible for the decreased efficiency of the cells (impaired disequilibirum), the increased risk for carcinogenesis and various other diseases claimed by the present invention (see below).

Alimentation and immobility in industrial societies is the most common cause of AS. Genetic predisposition is the second most important factor. The central role of the LDL receptor- apoA/E interaction was first appreciated in familial hypercholesterinemia (FH). LDL receptors in FH homozygotes cannot bind appropriately LDL-apoA/E. Ch accumulates in the circulation. This excess in circulating Ch causes AS, that begins in early childhood.

Orally administered Amp in humans normalizes circulating serum Ch within several weeks. The drug improves the Ch-uptake in the cell and the turn-over in the membrane. These results also indicate that Amp is effectively resorbed from the gut and exhibits a pronounced anticholesterolemic effect. Thus, Amp is a potent anticholesterolaemic drug and due to its safety profile should be used in early chronic treatment of AS.

Ch metabolism is ubiquitous. It affects steroid hormone metabolism and the bile production. Therefore, Amp can also be used in the treatment of following indications:

b) Alzheimer's Disease (Alz)

The apoE4 allele is significantly associated with Alz. This

lipoprotein is involved in Ch metabolism.

It could be shown that prolonged treatment with oral Amp can improve the symptoms of patients with Alz disease.

c) Prostatic Hyperplasia

old dogs with benign prostatic hypertrophy treated with oral Amp produced marked reduction in the texture and volume of the glands. Microscopic biopsies exhibited marked histologic improvements such as reduce congestion, granularity and papillations. These results suggest that polyenes are active for the treatment of prostate hyperplasia by the oral route. The effect is most probably due to a stimulation of the gland and improving the hormone production.

Polyenes can also be effective in the treatment of prostatic hyperplasia. Three male patients with prostatic hyperplasia and impaired urinary flow were treated with oral Amp for about three months. The size of the prostatic gland decreased dramatically after 8 weeks of treatment and the urinary flow markedly improved.

d) Gallstones

Amp stimulates the production of bile acids which are involved in the resorption from the gut. In a female patient with verified gallstones, a control sonography 2 months after beginning with oral treatment of Amp confirmed the disappearance of the gallstones. This was associated with a dramatic improvement of the symptoms.

e) Infertility:

Amp improves the homeostasis of Ch metabolism. Ch is the precursor of many steroid hormones. In female patients, treated with Amp we observed a dose-dependent increase in the vaginal discharge, probably due to enhancement of the prostaglandin

sysnthesis. A direct effect is also assumed.

f) Acne:

Patients with acne, including patients with acne conglobata were treated with Amp. There was a dramatic improvement in all patients. Inflamed efflorescences in acne conglobata were cured within 3 to 4 days of treatment. Acne vulgaris improved dramatically within 24 to 48 hours of treatment.

5. The Use of Amp in the Treatment of Cancer

Until now, there has been no established theory of carcinogenesis. In the context of this invention, a novel bioenergetic interpretation of carcinogenesis is suggested which explains the anticancer effects of Amp and other polyenes.

The transformation of cells into cancer cells occurs under the bioenergetic constraint to improve their impaired disequilibrium. Treatment with Amp means additional stimulation of all tissue cells that decreases the bioenergetic constraint of transformation. In addition, Amp stimulates the immune system which cooperatively eliminates carcinogenic cells. Amp enhances the uptake of cytostatic drugs in various cell cultures. Therefore, in this particular condition, a combination of polyenes with cytostatic drugs may be indicated.

Amp may be applied for treatment of the following cancers:
All kind of leukaemias: acute lymphatic leukaemia (ALL), acute myelogenous leukaemia (AML), chronic lymphatic leukaemia (CLL) chronic myelogenic leukaemia (CML), hairy cell leukaemia

Solid cancers such as (but not exclusively): breast cancer, choriocarcinoma, embryonal rhabdomyosarcoma, Ewings's sarcoma, Hodgkin's disease, lung, small cell (oat cell) carcinoma, other histologic types of lung carcinoma, Non-Hodgkin's lymphoma (Burkitt's lymphoma), diffuse large cell lymphoma, osteogenic

sarcoma, testicular cancer, prostatic cancer, cancer of the gastrointestinal tract (e.g. gastric carcinoma, colorectal carcinoma), gallbladder cancer, cancer of bile duct, cancer of urinary tract, renal cancer, pancreatic cancer, liver cancer, Wilm's tumor, adrenocortical carcinoma, brain cancer (e.g. glioblastoma, medulloblastoma), cervical carcinoma, uterine adenoma and carcinoma, cancer of the adnexes (e.g. ovarian cancer), endometrial cancer, head and neck cancer, islet cell carcinoma, thyroid neoplasm, Kaposi's sarcoma (AIDS-related and non-AIDS-related), mucosis fungoides, neuroblastoma, skin cancer, etc.

6. The Use of Amp in the Treatment of Peptic Ulcers, other Ulcers of the Mucosa of the Intestinal Tract, and Inflammations of the Mucosa of the Gastrointestinal Tract

Amp may be applied in the treatment of peptic ulcers (gastric ulcer and duodenal ulcer), other ulcers of the mucosa of the intestinal tract (e.g. colitis ulcerosa, stomatitis, etc.); and inflammations of the mucosa of the gastrointestinal tract (gastritis duodenitis, colitis, fistula etc.).

Ulcers and mucositis are the result of distorted balance between the aggressive factors (gastric acid, pepsin, etc.) and the mucosal defence or mucosal resistance to ulceration. Gastric mucus is the main component of mucosal defence. Various prostaglandins and especially those of the E series increase the gel thickness of the mucus and the production. Interruption of the gastric mucosal barrier in association with salicylate or ethanol ingestion causes hemorrhagic erosive gastritis. Amp stimulates prostaglandin synthesis, especially the production of prostaglandin E and thromboxane B2, by stimulating the phospholipid metabolism in general and by inducing the enzymes, cyclooxygenase and phospholipase A2, in particular.

In accordance with the present invention it was observed that Amp also enhances the production of gastrointestinal mucus. Maintenance of normal mucosal production is an essential

component of gastrointestinal resistance to injury. In the patients that were treated according to the invention with oral Amp, an increased production of the mucus was observed within the first two weeks. Dechallenge-rechallenge tests confirmed that the enhanced mucous production is directly related to the administration of oral Amp. In patients with chronic duodenal ulcers and gastritis, the symptoms improved under Amp treatment.

Mucosal lesions in the mouth can be effectively treated with topical Amp. Patients with subprosthetic stomatitis and mucosal inflammation around the dental roots were treated with a preparation of mucosal adhesive-paste. Pain and inflammation disappeared within 24-48 hours.

7. The Use of Amp in the Treatment of Osteoporosis and Other Degenerative Diseases

Osteoporosis can be idiopathic, but most often it is age-dependent. In postmenopausal women, in whom estrogen is deficient, osteoporosis regularly occurs. Osteoporosis is commonly associated with Cushing's syndrome. Glucocorticoids may potentiate the effects of the calcium-regulating hormones, parathormone and vitamin D (1,25(OH)2D), or counteract them, e.g. by inhibiting calcium absorption from the intestines. This dual character of Ch-derived hormones such as the steroid hormones, which is also typical for Ch (Ch has been defined as the "Janus" molecule) has not been adequately explained until now.

Ch regulates the dielectric properties of the membrane. This molecule determines the insulating properties of biologic membranes. So do steroid hormones. While Ch is electroneutral, i.e. its molecule has no dipole moment, all steroid hormones and physiological Ch-derivatives have a more or less pronounced dipole moment (e.g. testosterone, aldosterone, estrogen, gestagen, vitamin D, etc.). Thus, they increase the conductivity of the membrane in physiological concentration, by

replacing Ch. There is a very narrow range of homeostasis within which this can be done. Parenteral glucocorticoids are given in much higher concentration than normally observed in the organism. They excessively substitute Ch from biologic membranes and impair profoundly the Ch homeostasis and the function of the integral proteins. This depresses the cell metabolism. An excess in Ch (aging, AS, etc.) or a deficiency of membrane Ch (e.g. high levels of glucocorticoids) lead to an impairment of the dielectric milieu. For instance, osteoporosis is associated with diabetes mellitus. This means that the function of insulin, that stimulates unspecifically all cells by depolarazing (=activating) them and promoting glucose transport in the cell (Na^+-glucose symport during depolarisation, see above), is impaired in the periphery. One speaks often of peripheral resistance to insulin. The reason is either too much Ch (aging) or insufficient amounts of Ch in the membrane (e.g. Cushing's syndrome during glucocorticoid treatment). There has been no consistent explanation of these phenomena so far. Vitamin D which is central to calcium resorption and incorporation in the bone is also a derivative of Ch. 1,25(OH)2D represents a potent polyene that stimulates the cells by depolarizing them. It has an universal stimulating effect, similar to that of Amp. Vitamin D deficiency causes osteomalacia and osteoporosis. Substitution with vitamin D metabolites can treat osteoporosis as can substitution with estrogen in menopausal women. Treatment with Amp can improve osteoporosis by enhancing resorption of calcium from the gut and its incorporation in the bone and by stimulating the turn-over of bone tissue that is regulated by depolarizing physiological factors.

8. <u>The Use of Amp in the Pre-Treatment of Transplantation Patients</u>

Transplantation patients can be treated with cell homogenates from the donor or a coctail of corresponding HLA-alleles to "educate" the immune system of the host to tolerate the

HLA-allele of the donor. Simultaneously with this induced tolerization, the patient can be treated with Amp as a potent APC-stimulator to enhance the development of allo-tolerance in the host to the future transplant. Treatment must begin at least one year prior to transplantation to have enough time to boost the immune system of the host towards transplant-tolerance. This is feasible as hosts have to await a long time for transplantation.

III. Galenic Preparations for Amp

Depending on the specific indication Amp will be administered orally, topically or intranasally, as for example by inhalation.

The oral daily dose of Amp will be in the range of about 1 - 200 mg/kg body weight, preferably about 2 - 50 mg/kg body weight depending on the indication and the state of the disease. The total daily dose will be thus in the range from 100 mg to maximally 5 g. The maintenance daily dose of Amp is between 200 mg and 2 g. The frequency of application will be 1 to 6, preferably 1-4 times per day. 1 mg Amp corresponds to 5000 I.E.

Oral preparations of Amp will include tablets, capsules, lozenges, and powder for emulsions, and solutions (suspensions). Suitable preparations will include the pure active drug and a minimum of other necessary ingredients. Other active ingredients will not be used. For example, tablets may contain other ingredients such as ethyl cellulose, lactose, maize starch, magnesium stearate, talc, saccharose, soft paraffin, gelatin, wax, vanillin, etc. Lozenges may include ingredients such as flavours, d-mannitol, polyvinyl alcohol or other alcohols, magnesium stearate, talc, etc. Capsules will contain preferentially only the drug as powder, while other ingredients, if necessary, will be reduced to a minimum. Conventional capsules which are not dissolved by gastric juice

are preferred. In emulsions with Amp appropriate emulsifiers will be used. For solutions, adequate solvents can be used such as dimethylsulfoxide (DMSO).

Topical applications will include between 5 - 100 mg, (preferably 20 - 40 mg) Amp per gram of ointment. The frequency of application will be PRN. Suitable topical preparations will include creams, ointments, gels and pastes and solutions (suspensions). For example, the following ingredients may be used: soft and liquid paraffins, cetyl or stearyl alcohols, other alcohols, stearic acid, sorbic acid, sodium hydroxide, propyl and methyl hydrobenzoates, propylene glycol, glycerylmonostearate, scents, water, etc.

Inhalations with aerosol and nasal spray will be performed with inhalation solutions. The concentrations of Amp will be between 2 and 200 mg/ml, preferably 10-20 mg/ml

EXPERIMENTAL PART

Example 1: Treatment of HSV-Infection

Six patients with labial HSV infections were treated topically with Amp paste (20 mg Amp per g paste) shortly after exacerbation. There was a rapid improvement within 24-48 hours. Repeated application of Amp improved the effect.

Example 2: Treatment of Recurrent Aphthous Stomatitis (RAS)

Seven patients with minor and major RAS were treated with a mucosal-adherent preparation (paste) of Amp (20 mg per g). Applications were recommended every two hours in the acute phase and less frequently thereafter. Pain was relieved immediately after the first application. Depending on the diameter of the lesion, RAS disappeared within 24 to 48 hours. None of the patients experienced RAS for more than 4 days.

Example 3: Treatment of Rheumatiod Arthritis (RA)

Five patients suffering from RA were subjected to a long-term treatment by oral application of Amp. A mean daily oral dose of 1000 mg was applied. Positive effects occurred at as early as 3 months, but usually 6 months to 1 year after the beginning of the treatment, depending on the stage of the disease.

Example 4: Treatment of Gluten-sensitive Enteropathy

Two patients with gluten-sensitive enteropathy were treated for about 6 months with oral Amp (700-900 mg). A pronounced improvement of the symptoms was observed after 3 months.

Example 5: Treatment of Neurodermatitis

Five children with atopic neurodermatitis were treated topically with Amp paste (20 mg per g). Recurrent exacerbations were cured within 3 - 7 days, depending on the severity of the lesions. Untreated lesions did not resolve or resolved very slowly.

Example 6: Treatment of Postmenopausal Syndrome (PMS)

Three patients with postmenopausal syndrome (PMS) was treated with oral Amp (ascending dose from 700 mg to 1 g daily) for several months. The patients condition substantially improved during treatment. First effects were observed in the patient after 4 weeks. Proliferous vaginal serous discharge occurred after 2 weeks in the female patient that normalized to pre-menopausal levels in the course of the study. Dechallenge and rechallenge of Amp treatment for 1 week lead to a prompt interruption of the vaginal discharge.

Example 7: Reduction of Serum Cholesterol (Ch) in Humans

In the SDD trial a selected sample of hospitalized patients with elevated Ch and a known diagnosis of AS were screened for the Amp-lowering effect. There was a marked reduction of Ch plasma levels after 4 to 6 weeks. Amp also decreased plasma

levels of Ch in six ambulatory patients with AS after one to two months of oral treatment with 1g daily dose of Amp.

Example 8: Treatment of Prostatic Hyperplasia (Adenopathy)

One male patient with prostatic hyperplasia and impaired urinary flow was treated with oral Amp (1 g daily dose) for about three months. A rapid subjective improvement in the urinary flow was observed. Manual examination revealed a reduction in the size of the gland.

Example 9: Treatment of Gallstones

Twq elderly female patients with verified gallstones were treated orally with Amp (1 g daily dose). A control sonography 2 months after beginning of the treatment confirmed the disappearance of the gallstones. Symptomatically, there was also a dramatic improvement.

Example 10: Treatment of Acne

Eight patients with acne, including 2 patients with acne conglobata were treated. Amp cream (20 mg Amp per g) was applied topically several times during the day and before going to bed. There was a dramatic improvement in all patients.

Example 11: Stimulation of Increased Production of Mucus

In most patients treated with oral Amp in the above-mentioned trials (mean daily dose of 1 g Amp) an increased production of the mucus within the first two weeks after beginning of the treatment was observed. Dechallenge-rechallenge tests confirmed that the enhanced mucous production is directly related to the administration of oral Amp. In one patient with chronic duodenal ulcers and gastritis, the symptoms improved during a gastric crisis when she began Amp treatment.

Example 12: Treatment of Mucosal Lesions

Eleven patients with subprosthetic stomatitis and mucosal inflammation around the dental roots were treated with a preparation of mucosal adhesive-paste. The paste (20 mg Amp per g) was applied topically. Pain and inflammation disappeared within 24-48 hours.

Example 13: Treatment of Peptic Ulcers and Gastritis/Duodenitis

Two patients with gastric or duodenal ulcers were treated with Amp (1g daily) for 8 weeks and their symptoms were relieved.

Example 14: Treatment of MS

One patient with MS was treated with Amp (1 g daily) and their symptoms improved.

Example 15: Treatment of Trauma Patients with a Potential for Sepsis

Approximately 400 patients with severe trauma were treated either with Amp or Nys (approx. 500mg, 4 times a day)and their mortality was reduced. Further analysis of the laboratory data clearly indicated that the drugs were largely resorbed from the gut and exhibited systemic effects.

Example 16: Treatment of Patients with Ulcus Cruri, Decubitus and Burns

Patients with ulcus cruri (3), decubitus (4) and burns (7) were treated topically with Amp. The the lesions healed rapidely. (20 mg/g of cream, PRN)

Example 17: Peripoerative Prophylaxis

More than 100 patients received Amp (approx. 1g)for perioperative prophylaxis and the p.o. infection rate decreased from 2 to 8% to virtually zero.

M/35088

CLAIMS

1. The use of Amphotericin B or a functional derivative thereof for preparing an orally, intranasally or topically administrable pharmaceutical composition useful for the stimulation of bodycells of a human patient.

2. The use of claim 1 wherein modulation of the plasma electrochemical potential (EP) induces the bodycell stimulation.

3. The use of claim 2, wherein modulation is caused by the depolarization of the cells.

4. The use of claim 3, wherein energy transduction accross the plasma membrane is elicited in the process of cell depolarization.

5. The use of one of the claims 1 to 5, for preparing a pharmaceutical composition useful for treatment of a patient suffering from at least one of the following diseases:
   a) viral or bacterial infections,
   b) diseases associated with an impaired function of MHC class I and II and/or other integral proteins,
   c) cholesterol associated diseases,
   d) cancer,
   e) ulcers,
   f) degenerative diseases, or

   useful in wound-healing.

6. The use of claim 5, wherein the viral infection is caused by an infection with a virus selected from picornaviridae, caliciviridae, togaviridae, flaviviridae, coronaviridae, rhabdoviridae, filoviridae, paramyxoviridae, orthomyxoviridae, bunyaviridae, arenaviridae, reoviridae, retroviridae, hepadnaviridae, parvoviridae, papovaviridae,

adenoviridae, herpesviridae, poxviridae, Epstein-Barr virus, and cytomegalovirus.

7. The use of claim 6, wherein the virus is selected from HIV-1, HIV-2, HTLV-I, HTLV-II, Hepatitis-A-Virus, Hepatitis-B-Virus, Herpes-Simplex Virus, Herpes-Zoster Virus, Vesicular-Stomatitis-Virus, Influenza Virus, Reuscher-Leukemia Virus, Sindbis-Virus, Vaccinia Virus, or a virus triggering Recurrent Aphthous Stomatitis.

8. The use of claim 5, wherein the bacterial infection is caused by a gram-negative, gram-positive, aerobic or anaerobic bacterium.

9. The use of claim 5, wherein the disease is associated with an impaired function of MHC class I and II and/or other integral proteins and selected from diseases of the bone tissue, gastrointestinal diseases, hematologic diseases, skin diseases, endocrine and genetic disorders, neurologic disorders, renal disorders, infections, and genetic disorders of lymphokines, complement, other humoral factors and receptors.

10. The use of claim 9, wherein the disorder is selected from rheumatoid arthritis, food-sensitive enteropathy, neurodermatitis, chronic fatigue syndrom, multiple sclerosis, and Alzheimer disease.

11. The use of claim 5, wherein the cholesterol associated disease is selected from, atherosclerosis, Alzheimer disease, prostatic hyperplasia, postmenopausal syndrome, gallstones, infertility, and acne.

12. The use of claim 5, wherein the patient suffers from a solid cancer or leukaemia.

13. The use of claim 12 in combination with at least one additional cytostatic agent.

14. The use of claim 5, wherein the patient suffers from, gastric ulcer, duodenal ulcer, ulcers of the mucosa of the intestinal tract, inflammations of the mucosa of the gastrointestinal tract, decubitus, ulcus cruri.

15. The use of claim 5, where the patient suffers from lesions of the skin, burns and wounds.

16. The use of claim 5, wherein the patient suffers from osteoporosis.

17. The use of claim 5 for the prevention of postoperative infections, or for treating sepsis.

18. The use of claim 5, for enhancing the allo-tolerance of a patient to a future organ transplantate.

****

Patents Act 1977 Examiner's report to the Comptroller under Section 17 (The Search report)	Application number GB 9412983.0
Relevant Technical Fields (i) UK Cl (Ed.M) A5B (BHA, BJA) (ii) Int Cl (Ed.5) A61K 31/35	Search Examiner J F JENKINS Date of completion of Search 27 SEPTEMBER 1994
Databases (see below) (i) UK Patent Office collections of GB, EP, WO and US patent specifications. (ii) ONLINE DATABASES : WPI, CAS-ONLINE	Documents considered relevant following a search in respect of Claims :- 5(a), 6, 7, 8, 17 AND 18

Categories of documents

X:	Document indicating lack of novelty or of inventive step.	P:	Document published on or after the declared priority date but before the filing date of the present application.
Y:	Document indicating lack of inventive step if combined with one or more other documents of the same category.	E:	Patent document published on or after, but with priority date earlier than, the filing date of the present application.
A:	Document indicating technological background and/or state of the art.	&:	Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages		Relevant to claim(s)
X	EP 0542630 A2	(LAB MAYOLY SPINDLER) - see Claims 9 to 14	5(a), 6-8
X	WO 93/03737 A1	(LIPOSOME TECHNOLOGY) - see Claim 5	5(a), 6 and 7
X	WO 91/13595 A1	(THE SECRETARY OF THE ARMY, USA) - see page 7 line 23 to page 8 line 2 and lines 7 to 19	5(a) and 8
X	WO 89/03673 A1	(MEDIPRO) - whole document	5(a) and 8
X	Chemical Abstracts 113 : 144908 & J. Med. Virol. 31(2), pages 155-60 (1990) (J M CRANCE et al)		5(a) and 6
X	Chemical Abstracts 102 : 145993 & Mol. Genet. Mikrobiol, Virusol. 5, pages 41-6 (1984) (M A SHNEIDER)		5(a), 6 and 7
X	Chemical Abstracts 102 : 39536 & Antiviral Res. 4(5), pages 231-44 (1984) (B ALARCON et al)		5(a), 6 and 7

Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).